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Dahi (an immunonutrient) in India Culture

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ABSTRACT

Indian dahi, its history of origin, uses and benefits are reviewed. Studies were undertaken to observe effects of fermented milk in control of diarrhoea in children. India with high prevalence and severity of malnutrition, overpopulation and mainly vegetarian society are in need to develop protein sources with richness of minerals and vitamins; thus dahi (made with- lactobacillus bulgaricus and streptococcus thermophilus) and other vegetable source Berseem leaves (Trifolium Alexandrium) were studied in children patients suffering from protein energy malnutrition needing hospitalization, for acceptability and improvement in health i.e. appetite, weight gain, increase in haemoglobin. Study patients had clinical or laboratory evidence of infection, but only 32% patients were CRP positive (blunted response). The T cell lymphocytes CD3, CD4 and CD8 were in normal range and supplementation did not alter the counts. The baseline cytokine levels (TNFα, IFNγ, IL-10 and IL-4) were high in malnourished children. On feeding dahi and LPC diets the serum proinflammatory (TNFα, IFNγ), and anti-inflammatory (IL-10) cytokine levels, increased. The increase in IL-10 was higher in children receiving dahi diet. On comparing dahi diet against milk diet IL-1, IL-6 levels increased significantly on day 15th and at 6 weeks ($p<0.001$), in both the groups. However, IL-10 showed rise on day 15th and 6 weeks on dahi diet, in contrast milk diet patients showed fall on 15th day with subsequent rise at 6 weeks. The mean initial absolute lymphocyte counts were 3707±1551 and 4553±1776/ µl on dahi and milk diets, after 6weeks of treatment the corresponding values increased to 6312±1937 and 3493±1418 µl, respectively, ($p=0.004$). Similar, trend was observed for CD3+, CD4+, CD8+, CD19+ and CD56+ cells in two treatment groups. These observations demonstrate that dahi has immunonutrient properties.

KEYWORDS: Dahi, Fermented milks, Milk, Berseem, Diarrhoea, Protein energy malnutrition, Lymphocytes, Cytokines, Immunonutrient.

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INTRODUCTION

According to some historical texts, dahi / yogurt is believed to have been eaten as far back as 10,000 to 5,000 BC. In the Indian subcontinent- The child-god Krishna and his friends used to form human pyramids to break pots hung from the ceilings of neighborhood houses, in order to steal dahi and butter. This was in Vrindavana (Mathura)**,** Uttar Pradesh, India, where Krishna was brought up. Lord Krishna was born > 5252 years ago on 18th July 3228 BC. In Maharastra, Janmashtami is celebrated as 'Dahi Handi (*dahi*: curd, *handi*: earthen pot)'. It is organized roughly every August around Lord Krishna's birthday. The festival Gokulashtami, known as Krishna Janmashtami in the rest of the country, is the celebration of Krishna's birth and 'Dahi Handi' is part of it. The event involves making a human pyramid and breaking an earthen pot filled with milk/ curd/ or butter, fruits and water which is hung at a convenient height, thus imitating the actions of child God Krishna.

- Dahi is one of the five elixirs (Panchamrita) often used in Hindu rituals. In many parts of Indian meals, often conclude with dahi.
- Ayurvedic literature 600 AD mentions dahi for treatment of diarrhoea. The fat free curd (Butter milk) orally as well as enema was given to treat chronic gastrointestinal disorders e.g. colitis, chronic diarrhoea. etc. Dahi is also recommended for skin care, application softens skin, removes dead cells, possible effect of lactic acid. .

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- European encounter with yogurt/ dahi occurs in French clinical history: Francis- I suffered from severe diarrhoea which no French doctor could cure. His ally Suleiman the Magnificent sent a doctor, who allegedly cured the patient with dahi/ yogurt.

Dahi is the one indigenous ingredient that unites Indians in north- lassi and raita, south- dahi-rice or just dahi and in east - misthi dahi (sweet dahi). Meats are marinated in dahi by all communities, the food historian [1] points out that dahi -based marinates are mentioned in the Arthashastra, the importance of dahi in Indian cuisine dates back to prehistoric times. The Rig Veda mentions a dahi rice dish. The first references to 'Shrikhand' came around 500 BC. By 1000 AD, dahi was regularly used in Indian meals; it is almost as old as India itself [1]. The medical benefits of Indian dahi have been reviewed recently [2]. Dahi in Indian subcontinent is a traditional yogurt or fermented milk The earliest dahi/ yogurts were probably spontaneously fermented by wild bacteria lactobacillus delbrueckii subsp. bulgaricus prepared from cow's milk, and sometimes buffalo milk, or goat milk. The word *curd/ dahi* is used in Indian English to refer to naturally - home made yogurt, while the yogurt refers to the pasteurized commercial variety. Preparation-dahi is made by bacterial fermentation of milk. In this process lactose in milk is converted into lactic acid by several probiotic microorganisms. The species involved in the fermentation depend on the temperature and humidity of the environment, and may include *Lactococcus lactis*, *Streptococcus diacetylactis*, *Streptococcus cremoris*, *Lactobacillus delbrueckii subsp. bulgaricus* and *Streptococcus thermophilus*. After the starter is made, or saved from previous batch of dahi, milk is boiled and cooled. In a separate bowl curd is mixed with its whey, and then mixed together with the milk. It is then left to sit covered/ undisturbed for 5 to 10 hours. Dahi starter is sometimes made with dried red chillies (or their stems) in hot milk. Milk is boiled and then allowed to cool for a while. When lukewarm, dried chili peppers or their stems are added. The reason for this tradition is that dried chilies are rich in a type of lactobacilli, the bacteria that helps in fermentation of milk to form curd. These practices can also be applied for making curd from milk substitutes, such as milk prepared from soy, almond, cashew, or coconut.

REVIEW OF STUDIES

- **Study 1. To find out the effect of fermented milk preparations on control of acute diarrhoea in children.**

  Lactobacillus strains have been found to promote recovery from acute diarrhoea in children, mainly non bloody and non mucoid. The effect was specifically observed for reducing duration of acute diarrhoea, control of relapsing clostridium difficile colitis, antibiotic induced gastro-enteritis and even in diarrhoea due to vibrio cholerae and enterotoxigenic E coli. Lactobacillus strains have also been found effective in improving immunogenicity of Rota virus vaccine, and shedding of Rota virus in diarrhoea. A specific strain L.casei DN-114001 was able to protect mice against infection by Rotavirus. The results of the present study with L.casei DN-114001'Actimel' and dahi (NDRI) showed that these fermented milks controlled diarrhoea in both the hospital and the community studies, earlier by 0.6 and 0.5 day, respectively in those receiving 'Actimel'. Similarly, diarrhoea improved by 0.3 and 0.2 day in the group that received dahi (NDRI) $P<0.001$ (Table 1). Families using dahi regularly showed reduction in diarrhoeal episodes by 40% in children 6 month to 5 years of age during 3 months follow up in the community [3, 4].

- **Study-2. Countries like India with high prevalence and severity of malnutrition and mainly vegetarian society are in need to develop protein sources with richness of minerals and vitamins to control protein energy malnutrition with associated micronutrient deficiencies.**

  Fermented milks have shown immunonutrient properties, thus can be considered in the treatment of malnutrition (Protein energy malnutrition/ PEM), which besides malnutrition has immune disturbances and infections. Studies [5] have , reported an increase in the pro-inflammatory cytokine IFNγ in malnourished children on feeding yogurt-based diet. Similarly, oral administration in mice increased: a) number of immunoglobulin A producing plasma cells, b) phagocytosis, and c) the proportion of T lymphocytes and natural killer cells [6,7] .

PEM- deficiency of dietary protein or amino acids has long been known to impair immune functions and increase the susceptibility of animals and humans to infections. Protein malnutrition reduces/alters concentrations of most amino acids in plasma, erythrocytes, leucocytes and other body fluids: a) free alpha amino nitrogen was first to fall in leucocytes, being a progressive fall with severity of PEM [8, 9]; b) concentration of glutamic acid in erythrocytes increases suggesting intracellular production [10] , c) the ferritin showed early progressive fall in saliva and serum in PEM-I (early malnutrition) and in PEM grade III, the mean ferritin value was 3.28 ± 0.75 ug/L, only as compared to 169.3 ± 21.9 ug/L in normal children [11]. It may be possible that these children with PEM also had associated anemia [12]. Studies indicate an important role for amino acids in immune responses by regulating: (i) the activation of T lymphocytes, B lymphocytes, natural killer cells and macrophages; (ii) lymphocyte proliferation; and (iii) the production of antibodies, cytokines and other cytotoxic substances [13].

Researchers have shown that Indian dahi has total viable bacterial counts of $5x10^7$ to $5x10^8$ /ml; of these 10^7 to 3.5x to 10^8 /ml are lactobacilli, yeast and mould 700-5000, coliform 0-100 and spores 0-30/ml [14, 15]. The bacterial content and composition of dahi has been varying from place to place, some comparative data are summarized in Table 2.

Dahi was chosen as a test food in PEM for studying immunonutrient properties. The commonest bacteria in Indian dahi were lactobacillus bulgaricus and streptococcus thermophilus, being present in 80% or more samples. Dahi for the present studies was prepared using these bacteria.

b) Search for other food source(s)- To combat malnutrition and serve as additional weaning protein rich foods, so far the known leaves with such properties are: i) Tea leaves for decreased pro-inflammatory cytokine effect [16], ii) Azadirachta Indica (neem leaves) for enhancing TH -1 immune response [17], iii) Bersem Egyptian clover leaves (Trifolium Alexandrium) [18], iv) Drum stick leaves, not chosen in view of poor availability.

Berseem fresh leaves [18] with protein content 18-23% were considered. During this period (KNA) had an opportunity to examine a PhD thesis from the University of Jaipur, India in which Berseem leaves were fed to pregnant women, who showed acceptance and improvement in haemoglobin. In a tribal belt Berseem leaves fed to malnourished children also showed acceptance and benefit. Thus Berseem leaves were chosen to serve as control against dahi in children with malnutrition. M/s Dabur India Ltd, agreed to provide Berseem leaves in powder form, leaf protein concentrate (LPC), with protein content of 20% (prepared by ultra filtration and acid thermo coagulation); LPC- 100 g contained- 344 calories; fat 22.5 g, CHO 12.5 g, fibre 1 g, β-carotene 86700 µg, vitamin B complex- B1 0.5 mg, B2 0.5 mg, B5 24.2 mg, B6 1 mg, B9 330 mg, pantothenic acid 4.3 mg, vitamin C 2.2 mg, vitamin K 1 mg, Ca^{2+} 187 mg, P^{3+} 604 mg, Fe^{2+} 99 mg, Zn^{2+} 9 mg, Mg^{2+} 384 mg, Cu^{2+} 2.1 mg and K^+ 713 mg). Of the total protein intake for each patient 6 g were provided using either dahi or LPC (dried leaf powder in sachets, 3 such sachets provided 6 g of protein). Patients were required to consume LPC by mixing it in the dough used to make chapattis (bread) or in khichdi (a preparation of rice and lentil) or mixed with honey. Dahi was set in the hospital kitchen, using the starter provided by the NDRI, Karnal. 1 g of dahi contained 10^8 (CFU), each of l*actobacillus bulgaricus* and s*treptococcus thermophilus*. Dahi (100 g content- energy 60 cal, protein 3.1 g, fat 4 g, CHO 3 g, β-carotene 102 µg, B1 0.05 mg, B2 0.16 mg, B5 0.1 mg, B9 12.5 µg, vitamin C 1 mg, Ca^{2+} 149 mg, P^{3+} 93 mg, Fe^{2+} 0.2 mg, K^+ 130 mg, Na^+ 32 mg) was supplied to the subjects under refrigerated conditions.

Eighty moderate and severely malnourished children (grade II and grade III, age 1-5 yr (mean age 24.91 ± 11.13 months), requiring inpatient treatment for PEM and associated infection/infestation, during September 2003 to August 2004 received the WHO [19], recommended management and diet modified for local dietary habits, containing in addition either dahi or LPC, for a period of 15 days. Patients were also given oral albendazole, oral chloroquine. For infection children received parenteral ampicillin (100 mg/kg/day) and gentamycin (5 mg/kg/day). Clinical non-improvement or deterioration, after 48 hr of treatment warranted change to ceftriaxone (75 mg/kg/day) and amikacin (15mg/kg/day) or antibiotics as per sensitivity report. Only 32 patients in dahi group and 36 in LPC group completed the study for 15 days [20-22].

All children received a two-step mixed vegetarian diet from the hospital kitchen over 15 days, as per the recommended WHO guidelines [19].

Initial treatment phase (Days 1 to 7): Treatment of dehydration, correction of electrolyte imbalance, appropriate antibiotics for associated acute infections and initiation of feeding to provide about 75- 100 kcal/kg/day-contains 75 kcal of energy and 0.9 g protein per 100ml.

(Rehabilitation phase (Days 7 to 15): Increasing the calorie intake to 150-200 kcal/kg/day contains 100 kcal of energy and 2.9g proteins per 100ml.

Nutritional, immunological and haematological parameters were measured before and after supplementation and compared within the groups. The haemoglobin increased and serum ferritin showed a fall in the dahi and LPC groups, after supplementation ($P<0.001$). Though all patients had clinical or laboratory evidence of infection, only 22 (32%) patients were CRP positive, initially. Of these, 8 were in dahi group and 14 in LPC group. After supplementation, one in dahi and 5 in LPC groups remained CRP positive. Of these, 4 patients were not positive for CRP at the beginning of the study (blunting of response).

The mean T cell subpopulations (CD3, CD4 and CD8) counts in both the groups were within the normal reference range for age, with no change after treatment. However, there was increase in CD4: CD8 ratios in both the groups, after treatment.

The baseline cytokine levels (TNFα, IFNγ, IL-10 and IL-4) were high in malnourished children. Both the diets increased the serum proinflammatory (TNFα, IFNγ), and anti-inflammatory (IL-10) cytokine levels, after nutritional rehabilitation. The increase in IL-10 was significant in children receiving dahi. There was insignificant fall in IL-4 levels with both the diets. LPC in malnutrition showed comparatively better proinflammatory response and reduction in IL-4 as compared to dahi diet, which showed higher increase of anti-inflammatory IL-10 (Table 3) [22].

Protein Energy Malnutrition (PEM) is the most frequent cause of secondary immune deficiency in children with significant impairment of cell-mediated and humoral immune responses. Treatment of malnutrition should reverse the acquired immune dysfunction(s). WHO recommends [19] that children with severe acute malnutrition need to be treated with specialized therapeutic diets (F75 and F100 formula) alongside the diagnosis and management of complications during in-patient care. Recent studies [20-22] have shown that fermented milk (Indian dahi) in diet: a) prevented as well as controlled diarrhoea [3,4] and b) in moderate to severe malnutrition- cytokine levels showed an increase in pro-inflammatory (TNFα, IFNγ), anti-inflammatory (IL-10) but a fall in IL-4, with an increase in CD4:CD8 ratio.

These immunonutrient properties of dahi, in moderate to severe PEM were compared against the recommended WHO milk diet [23]. Study was conducted during December 2007 to November 2009. The methods used in earlier study [20] for patient's recruitment, diet, medicines and techniques used for blood collection and estimations were followed. 4.0 ml fresh venous blood was collected in plane vials and the serum was separated and stored at -20 º C. All patients received antibiotics for associated infections, if required according to the protocol [20]. Therapeutic WHO [19] diet for malnutrition modified/adapted to local dietary practices was given to both groups for a period of 15 days (Period of hospitalization). Group -A received diet including dahi and other group B received diet with milk. Both were prepared and supplied by M/s Mother Dairy, Delhi in coded containers of 90 gm each under refrigerated conditions and maintained in the hospital. However, the difference in consistency remained. Dahi contained 10^8 **c**olony forming unit (CFU) of *lactobacillus bulgaricus* and 10^8 (CFU) of *streptococcus thermophilus* per gm and other group received similar milk in diet. Each patient received 2 containers per day with lunch and dinner.

Clinical, immunological assessment, cytokines levels and immunophenotyping were done on Days1 and 15 of hospitalization. Patients were discharged after 15 days and were followed up in the outpatient services every week with repeat clinical immunological assessment and immunophenotyping at 6 wk. During this period they continued to consume dahi or milk in diet as before, served under refrigerated conditions by field workers.

Haematological parameters: haemoglobin, total leukocyte count and CRP. Immunological parameters: Interleukin-1, Interleukin-6, Interleukin-10 were estimated[20-22]. Absolute lymphocyte count, sub lymphocyte population (CD3+ cells, CD4+ cells, CD8+ cells, CD19+ cells and CD16+56 cells). T cell and B cell population estimation: 1 to 1.5 ml of venous blood in EDTA as anticoagulant, was transferred to M/s Vimta Labs, Cherlapally Hyderabad, A P, India (Delhi center). The T and B cell populations were estimated by BD FACS caliber flow cytometers using four colour cytometry (Immunophenotyping). T and B cell subpopulations were enumerated using Monoclonal antibodies directed against cell surface antigens. Quantification of subpopulation is done using liquid counting beads [23].

Twenty normal healthy controls between the age group 2 to 5 yr were also enrolled from the child health check up clinic and investigations were undertaken for haematological and immunological assessment. The values of the study patients were compared with the age matched healthy control. The biological reference intervals of various absolute lymphocytes cells/ µl are : a) lymphocyte 1392-3510 , b) CD3: 1010-2659, c) CD4 381-1170, d) CD8 108-845, e) CD19 98-842 and f) CD3-CD16+56 (NK cells) 90-590 cells/ µl.

The mean age of the study patients was 3.13± 0.88 years. The mean age of patients in group A (WHO-dahi diet) was 3.18± 0.92 yr and group B (WHO milk diet) was 3.09± 0.86 yr, being comparable. Patients were classified as having severe malnutrition, 25 with severe wasting (wt/ht <70%); 24 with severe stunting (ht/age < 85%) and 18 children had both. 5 children in group A and 3 in group B had oedema. The major associated ailments in these patients were acute gastroenteritis (53%), bronchopneumonia (38.7%) and pyoderma (8.1%). The clinical improvement was seen in 3 to 5 days in both the groups after appropriate therapy, with no significant difference between the two groups.

The initial weight values in groups A(dahi) and B(milk) were (7.8±1.4 and 6.7±1.7 kg, respectively; p=0.31). Both groups' showed significant increase in weight after 6 week of treatment, gains being 327 gm in group A as compared to 240 gm in group B ($p < 0.001$). The height was significantly higher initially in group A (79.6 ±6.9cm) as compared to group B (77.5±7.5cm) at the beginning of study. The gains at 6 week was 45 mm in group A and 40 mm in group B ($p<0.001$ for both).

The initial haemoglobin values were 8.6±1.5 and 8.8±1.7 gm/dl in groups A and B, there was fall by 0.5gm at 15 days in both the groups, with subsequent rise at 6 week being 0.33gm and 0.10gm/dl, respectively. Cytokines IL-1, IL-6 and IL-10 in age matched healthy children (n=4 at 2-3 yr, n=5 at 3-4 yr and n=11 at 4-5 yr of age) means were 10.6±5.6, 8.3±5.7 and 11.4±6.4 pg/dl respectively. The initial values for these interleukins were significantly higher in children suffering of protein energy malnutrition. On treatment IL-1, IL-6 levels increased significantly on day 15th and at 6 week ($p<0.001$), in both the groups. IL-10 showed similar rise on day 15th and 6 week on WHO-dahi diet, but there was fall on 15th day on WHO-milk diet with subsequent rise at 6 weeks. The rise in interleukin levels, during treatment were much higher on WHO-dahi diet as compared to WHO- milk diet (Table 4).

Absolute Lymphocyte counts- Initially 2 patients on WHO-dahi diet had counts of 1468 and 1483/µl which increased to 9173 and 5366/ µl after 6 week of treatment. The mean initial absolute lymphocyte counts were 3707±1551 and 4553±1776/ µl in WHO-dahi and WHO-milk diets, after 6weeks of treatment the corresponding values were 6312±1937 and 3493±1418 µl respectively. Thus WHO- diet with dahi showed increase and WHO milk diet decrease in counts post therapy, these changes being significant (p=0.004). Similar trend was observed for CD3+ CD4+ CD8+, CD19+ and CD56+ cells in two treatment groups .At 6 weeks differences in change for CD3+cells and CD4+ cells were significant p being p<0.003 and 0.001, respectively. The changes for CD+8, CD19+ and CD56+ were not significant (Table 5) [23]. Studies with fermented milk had also shown that immunopeptides increase the activity of the immune system cells, such as proliferation of lymphocytes, activity of NK cells, synthesis of antibodies and production of cytokines [24].

CONCLUSION

The restoration of CRP activity, increase in levels of cytokines and lymphocytes on WHO-dahi diet, and acceptability suggest its role as immunonutrient, compare to WHO milk diet for treating PEM. In addition LPC (leaf protein concentrate) performed better than dahi (fermented milk) group, TNFα, INFγ (proinflammatory) , can be chosen as additional protein source in food/ snacks to serve as an extra source of protein.

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Table I. Comparison between the three preparations studied.

Content	Ultra Heated Yogurt (g)	National Dairy Research Institute Karnal (NDRI) Dahi (g)	L.casei (g) (Actimel) Danone
Total solids	23	18	18.5
Fat	3	1.5	1.5
Sugar	11	9	9.2
Protein	2.8	2.9	2.8
Bacterial Profile	0	10^8 each of	10^8 each of
		Lactococcus lactis; Lactococcus lactis; cremoris; Leuconostac mesenteroides cremoris per g	Lactobacillus bulgaricus; Streptococcus thermophillus; Lactobacillus casei; DN-114001 per g

Table 2. Proportion of samples having bacterial presence in Indian dahi.

	Islamabad [14]	Bangalore [15]
Lactobacillus bulgaricus	86	83
Streptococcus thermophilus	80	85
Streptococcus lactis	74	7
Lactobacillus helveticus	34	5
Streptococcus cremoris	30	7
Lactobacillus casei	20	11
Lactobacillus acidophilus	14	-

Table 3: LPC (leaf protein concentrate) group performed better than dahi (fermented milk) group, excepting IL-10.

Groups/parameters	TN Fα	IF Nγ	IL-4	IL-10	CD4/CD8 ratio
Leaf protein (LPC) % change -32 patients	90.2	36.1	-66.9	7.81	26
Fermented milk (FM) % change -36 patients	38.5	31.9	-23.2	117.6	20.8

Table 4. Cytokine Il-1, IL-6 and IL-10 pg/dl, initial levels and during treatment at 15 days and 6 week in protein energy malnutrition.

Treatment groups	IL-1 mean± sd	Control IL-1 =10.6±5.6	IL-1 mean ±sd
A-WHO-dahi diet n=25 on 1 and 15 days, and 22 at 6 weeks.	Day-1 54.6±121(1.27±0.51) Day-15 96.9±160.7(1.47±0.59) Weeks-6 181.0±192.7(2.01±0.46)	B.WHO-milk diet n=24 on 1 and 15 days. And 20 at 6 weeks	Day-1 29.3±41.6(1.2±0.5) Day-15 71.6±135.9(1.43±0.56) Weeks-6 115.3±168.6(1.71±0.55)

	IL-6 mean ±sd	Control= IL-6 8.3±5.7	IL-6 mean±sd
-	Day-1 33.0±47.1(1.12±0.63) Day-15 78.5±79.8(1.49±0.71) Weeks-6 128.9±62.8(2.02±0.35)		Day-1 32.3±36.5(1.27±0.52) Day-15 45.0±49.0(1.38±0.56) Weeks-6 77.0±58.3(1.67±0.54)
	IL-10 mean± sd	Control IL-10 11.4±6.4	IL-10 mean±sd
	Day-1 20.1±19.8(1.1±0.31) Day-15 36.6±39.5(1.34±0.46) Weeks-6 64.0±48.8(1.65±0.42)		Day-1 23.2±37.3(1.14±0.42) Day-15 14.8±10.3(1.08±0.29) Weeks-6 36.6±34.2(1.33±0.53)

In parenthesis are the transformed log levels.

Table 5. Showing mean ± sd values of lymphocytes on day-1 and after 6 weeks of feeding dahi or milk in WHO diet in children with protein energy malnutrition.

WHO diet groups	Days	ALC(cells per µl) (absolute lymphocyte count) mean±sd	CD3+ cells mean±sd	CD4+ cells mean±sd	CD8+ cells mean±sd	CD19+ cells mean±sd	CD56+cells mean±sd
WHO dahi diet Change mean±sd	Day 1	3708±1551 (30.07%)	2156±724 (61.7%)	1842±1720 (28.1%)	838±309 (23.7%)	1164±748 (30.3%) Table	404±320 (11.2%)
	6wk	6312±1937 (36.9) +2549 ±2362	3793±1047 (63.2%) +1636 ±995	2167±760 (35.8%) +948 ±771	1236±414 (20.9%) +398 ±508	1314±798 (26.8%) +560 ±1564	678±379 (11.4%) +273 ±526
WHO milk diet Change mean±sd	Day1	4553±1776 (33%)	3517±1605 (59.9%)	1931.±1304 (34.5%)	1410±988 (26.2%)	1550±1850 (21.2%)	362±185 (11.7%)
	6wk	3494±1418 (32.4%) -835 ±1180 P<0.004	2897±1624 (58.8%) -620 ±869 <0.001	1696±1307 (32.6%) -235 ±451 <0.003	1320±879 (24.3%) -90 ±506 NS	1408±1816 (19.4%) -441 ±749 NS	339±154 s(10.4%) -103 ±186 NS

Values in paranthesis are the percentages of total leucocyte counts.